The Fatty Acid and Glyceride Structures of Indian Buffalo Milk and Depot Fats, and some Characteristics of Eastern Animal Fats

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(*Received* 27 *May* 1946)

This work describes the fatty acid and glyceride structures of Indian buffalo milk and depot fats. Only three other analyses of buffalo milk fat exist in the literature (Bhattacharya & Hilditch, 1931; Heiduschka & Cicekdagi, 1940), in only one of which was an examination made of the glyceride configuration, while no analysis of buffalo depot fat has so far been reported. The work below was therefore undertaken to indicate peculiarities, if any, in the make-up of the fats and general fat metabolism of the buffalo. Finally, data are presented to illustrate some striking peculiarities of Indian animal fats in both fatty acid and glyceride structures.

RESULTS

Fatty acid structures of buffalo milk fats

The four samples of milk fat used have been described elsewhere (Achaya & Banerjee, 1946); their analyses (by the ester-fractionation method of Smith & Dastur (1938) using an electrically heated column of the type described by Longenecker (1937)) and brief interpretative notes are included below for the sake of completeness. The glyceride structures outlined later appear however for the first time. Table 1 indicates these results.

Table 1. *The characteristics and composition of the Indian buffalo milk fats*

Sample	1	2	3	4
Origin	Agricultural College, Kirkee	Dinshaw Dairy, Bangalore	Satvirda Hills, Porbandar	Satvirda Hills, Porbandar
Feed	Pasture, groundnut cake, concentrates	Pasture, oil-seeds	Heavy cotton-seed feeding, little or no pasture	
Appearance	Excellent flavour and texture, bright yellow colour, fresh		Good flavour, small grains, hard, white in colour	Rancid, choking flavour, small grains, hard, white in colour
Noteworthy feature:	High R.M.	Normal R.M.	Low R.M.	Low R.M., rancid
R.M.	37·4	30·8	20·7	22·7
P.V.	1·9	1·2	0·6	0·8
i.v.	27·4	28·9	37·0	34·9
s.v.	227·3	223·7	212·6	216·7
Free fatty acids (as % lactic)	0·07	0·07	0·09	1·7
Acid (% by mol.)				
Butyric	15·4	13·5	11·5	10·1
Caproic	1·1	0·4	—	0·7
Caprylic	1·4	0·5	0·1	2·2
Capric	1·4	0·9	0·5	1·8
Lauric	1·9	2·4	0·8	2·6
Myristic	9·2	12·3	4·8	7·1
Palmitic	31·9	31·5	25·1	22·5
Stearic	12·5	10·1	19·0	16·8
as-Arachidic	0·1	—	1·1	1·0
Decenoic	0·1	0·1	0·03 } 0·1	0·3
Dodecenoic	0·1	0·1	0·05 }	0·2
Tetradecenoic	0·6	1·0	0·5	0·8
Hexadecenoic	3·0	3·0	2·9	5·1
Oleic	16 8	23·0	32·0	28·6
Octadecadienoic	1·2	0·4	1·0	0·2
as-Gadoleic	3·3	0·8	0·6	—

R.M. = Reichert-Meissl value.
P.V. = Polenske value.
i.v. = iodine value.
s.v. = saponification value.

The high content of lower saturated and unsaturated acids in sample 4 is no doubt due to the marked rancidity that had occurred, and the value of this analysis is only to confirm certain characteristics of its counterpart 3 from a cotton-seed feeding area, viz. high stearic and low myristic contents. These analyses also reveal for the first time the presence in Indian buffalo milk fat both of unsaturated acids lower than, and more unsaturated than, oleic acid. The linoleic contents generally tend to be extremely low.

There exists in general a marked inverse relationship between the lower acids, on the one hand, and oleic on the other, similar to the results of Smith & Dastur (1938) on inanition, and best explained by the theory of Hilditch & co-workers (Hilditch, 1940) of the mobilization of preformed oleoglycerides by the mammary gland. This is the more marked in that samples 1 and 2, of about the same iodine value, contain widely different proportions of oleic acid, with therefore differences in the linoleic acid percentages.

Comparison of the figures for fats 3 and 4 with the three other analyses extant (Bhattacharya & Hilditch, 1931; Heiduschka & Cicekdagi, 1940) indicates the existence of two main types of buffalo milk fat associated, respectively, with high palmitic and high stearic contents; moreover, the high stearic figure need not necessarily be associated with a low content of lower acids as in the present case. This suggests that the proportions of stearic acid are determined by a mechanism independent of that operating in fixing the proportions of oleic acid on the one hand and the lower acids on the other. Emphasis is laid on the high stearic rather than on the low palmitic acid content of these samples since the percentage of palmitic acid normally tends to constancy in milk fats and is low in this case as a mathematical consequence of the high stearic figure coupled with a high oleic percentage besides; moreover, Patel, Patel & Dave (1944) have shown that the buffalo appears to be much more susceptible to feeding vagaries than the cow under identical conditions.

The source of this high stearic acid is almost certainly the oleic or linoleic acid from dietary cotton-seed. It is, however, difficult to explain why other oils, e.g. soya bean, containing these acids in similar proportions, do not produce any untoward effect on the stearic acid content of milk fats.

Features approaching constancy in the above figures would appear to be the sum of myristic, palmitic and stearic acids, and the percentage of hexadecenoic acid; as criteria for the detection of adulteration in milk fat, routine isolation and estimation of these quantities would be almost impossible. The low linoleic acid content of these fats (qualitatively measured by the iodine and thiocyanogen values) could perhaps profitably be measured as a test for purity, especially considering the high content of the acid in the raw or even hardened vegetable oils used for adulteration in India.

Fatty acid structure of buffalo depot fats

These depot fats were collected from the local slaughter house and analyzed when quite fresh. It was thought desirable in the first instance to collect individual specimens of fat rather than a possibly more representative mixed sample. The results are shown in Table 2.

Sample B calls for comment on several grounds. It was bright yellow in colour, unlike the other specimens and of low i.v., though surprisingly of low melting point as well, due no doubt to a high palmitic content. Also, it contained substantial quantities of lauric acid, which, though normal in a western tallow (Hilditch & Longenecker, 1937), do not appear in the other specimens above. Even more surprising was the appearance of the unsaturated dodecenoic acid, the inclusion of which can be justified for many reasons: the low molecular weight (214·6) of the first unsaturated ester fraction, confirmed by the still low value (225·7) for the next; a progressive rise and fall (28·32, 34·46, 28·02, etc.) in the i.v. of these ester fractions, generally characteristic of the appearance and disappearance of such acids; and, finally, the fact that if the unsaturation had been calculated to tetradecenoic ester, the molecular weight of the saturated esters would have been so lowered as to necessitate provision for a C_{10} saturated ester, which, considering the non-appearance of even a C_{12} saturated acid in the other fats, might well have been rash.

The i.v. of these fats has fallen even below the extremely low values of the four Indian cow depot fats studied by Hilditch & Murti (1940), and this has been reflected, if only partly, in the slightly higher melting points of these fats as well.

The three fats analyzed clearly fall into two categories: samples A and C of high stearic content and sample B of high palmitic content. Hilditch & co-workers (Hilditch, 1940) had formerly believed, on the basis of a study of tallows of western origin, that a marked inverse oleic-stearic relationship obtained; palmitic acid under a wide variety of conditions tending to remain constant at $27 \pm 3\%$ (by mol.). After the study of the Indian cow depot fats, however, they postulated that in such extreme cases, where the stearic-oleic relationship could not be pushed so far as to exceed a certain stearic maximum, palmitic acid was augmented instead, the whole procedure being no doubt regulated by a certain degree of fluidity of the fat indicated by a melting point of about 50°. The melting points in the present instances are 1–2° higher, so that it is

Table 2. *The characteristics and composition of Indian buffalo depot fats*

Sample	A	B	C
Origin	Local slaughter house, mixed fat from two buffalo cows in milk, from back and dewlap respectively	Local slaughter house, from dewlap of buffalo cow in milk	Local slaughter house, from buffalo cow past milk, location uncertain
Probable feed	Pasture in large amounts, horsegram, wheat bran, cotton-seed in variable amounts		
Appearance	Tallowy odour, hard, very pale yellow	Tallowy odour, hard, bright yellow	Tallowy odour, hard, very pale yellow
Melting-point	51·4°	50·6°	52·1°
Solidification point	45·1°	44·2°	44·7°
$n_D^{60°}$	1·4486	1·4480	1·4487
S.E.	281·1	277·7	286·7
I.V.	26·4	23·8	26·8
Colour (Lovibond Y.U./g.)	1·6	5·0	1·6
Vitamin A	Blue colour with standard Carr-Price reagent hardly perceptible, little or none		
Free fatty acids (as % lactic)	0·08	0·08	0·1
Acid (% by mol.)			
Lauric	—	1·4	—
Myristic	3·9	7·4	4·4
Palmitic	33·4	45·6	33·5
Stearic	31·7	19·2	34·1
as-Arachidic	0·5	0·3	1·0
Dodecenoic	—	0·4	—
Tetradecenoic	0·4	0·8	0·5
Hexadecenoic	2·0	1·8	2·3
Oleic	27·8	22·3	24·1
Octadecadienoic	—	0·7	0·1
as-Gadoleic	0·3	0·1	—

conceivable that the stearic figure permitted could well be a few units higher. Such appears to be the case, for the highest figure here is about 5·5 units higher than the maximum for the cow depot fats.

The three main fatty acid components of depot fats are palmitic, stearic and oleic acids. While in milk fats, a balance of components following on the forced modification of normal milk fats as a result of feeding, e.g. cotton-seed meal, is brought about by large variations in the oleic acid content, this component could not in reserve fats be very markedly increased or decreased, since the latter presumably perform a much more specific function than the former in the animal body. Hence an inverse stearic-palmitic balance is all that is left if the body has to deal effectively with fat. Cotton-seed meal has been shown to have the effect of increasing stearic acid and of tending to increase the oleic content as well; while in milk fats the palmitic percentage was not unduly depressed, since a marked lowering of the lower fatty acid content took place. The same procedure is not here feasible, though evidences of its occurrence are visible in lower lauric and myristic contents in samples A and C: the only course open has been a lowering of the palmitic acid.

This leads to the view that the fat B represents the basic buffalo depot type and that A and C are modifications, probably as a result of cotton-seed feeding, of the former. It is at least suggestive that fat B should be markedly yellow in colour and that cotton-seed-fed buffaloes yield extremely white milk fats. The widespread use of cotton-seed meal as cattle fodder would account for the frequency of the latter type, as evidenced by two of the three cases above and three of the four samples of Hilditch & Murti (1940). Peculiarly enough, linoleic acid is present only in traces or not at all in these Indian animal depot fats, exactly as pointed out earlier for the milk fats, though the percentages of higher unsaturated acids appear normal.

Glyceride structures of Indian buffalo milk and depot fats

The fully saturated glycerides were estimated by the usual method of Hilditch & Lea (1927), and the proportions of the other glyceride categories calculated on the assumption that no tri-unsaturated glycerides exist. Samples 1 and 3 of milk fat and samples A and B of depot fat were used since they represent distinct types in their respective categories. The fatty acids of the fully saturated glyceride portion in the case of the depot fats were determined by ester-fractionation. Table 3 indicates the results.

Table 3. *Glyceride structures of Indian buffalo milk and depot fats*

% by mol. of	Milk fats 1	Milk fats 3	Depot fats A	Depot fats B
Fully-saturated glycerides	41·7	24·3	32·4	37·2
Mono 'oleo' disaturated glycerides	41·2	40·1	43·5	47·3
Di-'oleo' monosaturated glycerides	17·1	35·6	24·1	15·5
Total saturated acids	74·9	62·9	69·5	73·9
Association ratio	1·32	1·04	1·22	1·41
Fatty acid structure of fully saturated glycerides (% by mol.):				
Myristic			9·2	14·4
Palmitic			43·7	52·1
Stearic			47·1	33·5

While here, as usual, the association ratio increases with increasing total saturated acids and thus of fully-saturated glycerides, the values for Indian animal fats appear to be of the order of 0·1–0·2 unit less than for a corresponding sample of fat in the West. Indian sheep, goat and camel milk and body fats show analogous high association ratios (cf. Hilditch, 1940). Similarly the proportions of fully saturated glycerides corresponding to any definite percentage of total saturated acids are in these instances distinctly lower than in fats of western origin.

Here, as in western animal fats, the percentage of mono 'oleo' disaturated glycerides is relatively constant, even over so wide a range of type as the above samples. The proportions of such glycerides are however much greater than for western fats. The implications of the above observations will be stressed in the concluding section of this paper.

The fatty acid nature of the fully saturated part of the body fats confirm again the observations of Hilditch and his colleagues (Hilditch, 1940) that though the ratio of myristic and palmitic to stearic acid in the whole fat varies very widely, the ratio in the fully-saturated glycerides lies always between the limits 1:1 and 2:1.

Some characteristics of eastern animal fats

Attention has been called above to several unusual features in the fatty acid and glyceride structures of Indian animal fats. These may now be discussed in some detail.

The work of Ivanow (1922–3) has shown that plants which thrive in both hot and cold climates produce in the former case seed fats that are relatively saturated, no doubt because a certain specific and effective degree of fluidity of the fat must be maintained relative to the outside temperature. It would now appear that animal fats from countries of the East with hot climates also exhibit similar characteristics of saturation. Milk fats will first be considered and then the adipose tissue fats of animals. Empirical analysis of over 160 Indian milk fats indicated that the unsaturation varied for cow and buffalo samples from an i.v. of 24 to one of 40 (Achaya, Katrak & Banerjee, 1946). The extreme saturation of these milk fats compared to those of western origin which commonly exhibit iodine values of the order of 40, is very evident. There was a marked inverse relationship between Reichert-Meissl and iodine values in both cow and buffalo milk fat, but the iodine values of the former were about 3 units higher in each case than buffalo milk fats of the same Reichert-Meissl value.

Since the precursor of both milk and depot fat is almost certainly the neutral triglyceride fraction of blood, it may seem inevitable to find markedly saturated reserve fats as well in Indian animals. Such indeed is the case: four samples of Indian cow depot fats examined by Hilditch & Murti (1940) showed extremely low figures for i.v. (25·8 to 31·1) compared to western beef tallows (i.v. about 40); while the analyses of the three Indian buffalo depot fats recorded above reveal values lying between 23 and 27 which, though too small in number in either case for an unqualified opinion, appear to be of the order of 3 units less, as in the milk fats. The marked saturation of both types is again apparent.

In addition to these regional and species differences in the fatty acid assembly of Indian animal fats, there also appear to be striking peculiarities in their specific glyceride structures. Hilditch & his associates (Hilditch, 1940) have shown that if the fully saturated glyceride percentage of animal fats be plotted against the corresponding percentage of total saturated acids of the fat, the points lie on a smooth curve (curve 1 in Fig. 1) which cuts the saturated acid axis at a point corresponding to about 25% (by mol.) which is invariably the proportion of palmitic acid found in these fats. More generally, the palmitic acid contents tend to be 23 ± 3% (by mol.) in the milk fats and 27 ± 3% (by mol.) in the depot. If, however, similar points be plotted for the fats from Indian animals, it is found that they appear rather to lie on a well-defined line about 4 units (by mol.) below the former. This graph cuts the saturated acid axis (new curve 2 in Fig. 1) at a point corresponding to about 30% (by mol.). Strikingly enough, the proportions of palmitic acid found in these fats are of this order: to make a further particularization, the percentages are about 27 ± 3 for the milk and 31 ± 3 for the depot fats. These relationships are shown in Table 4 and Fig. 1.

In addition to these twelve cases worked out in full, data are available from fatty acid analyses on the palmitic acid contents of three more buffalo milk fats (one by Bhattacharya & Hilditch, 1931; one by Heiduschka & Cicekdagi, 1940; and sample

2 of the present series), and one more cow milk fat (Bhattacharya & Hilditch, 1931); and of two cow depot fats (Hilditch & Murti, 1940), one buffalo depot fat (sample C of the present series), and seven wild animal fats (Hilditch, Sime & Maddison, 1942). These fourteen cases tend to confirm the new glyceride relationships postulated above for eastern animal fats.

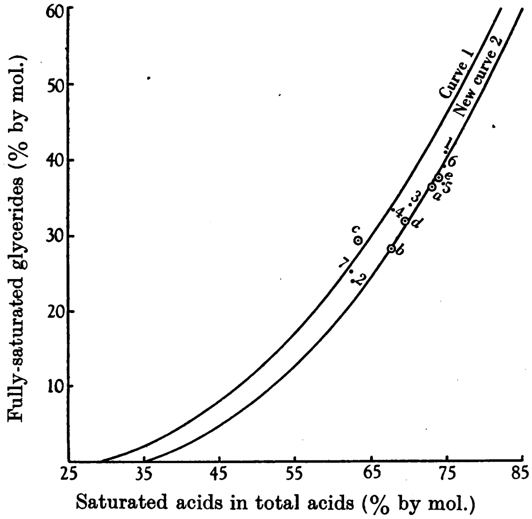

Fig. 1. Comparative glyceride relationships of western (curve 1) and Indian (new curve 2) milk and depot fats. Curve 1 from Hilditch (1940).

Of the five apparent exceptions to these twenty-six cases, the goat and sheep milk fats of Dhingra (1933) were from animals on a winter diet in a cold province like the Punjab and may indeed be said to support the contention that it is in a tropical climate that the above relationships would hold; the low palmitic content (20·2) of the sacred baboon fat of Hilditch *et al.* (1942) is no doubt due to the low content of total saturated acids (29·7) and very high unsaturation, and that (19·0) of the buffalo milk fat of Bhattacharya & Hilditch (1931) possibly to dietary cotton-seed, a fact supported by the high stearic figure accompanying. The high palmitic percentage of buffalo depot fat B of the present series has already been commented upon.

As a corollary to this lower content of fully saturated glycerides corresponding to any definite percentage of total saturated acids, Indian animal fats are also characterized by a higher, though still normally constant, content of mono-'oleo' glycerides than fats of European origin, and by higher association ratios, since the proportions of saturated acids in the mixed glycerides increase with respect to any definite proportion of unsaturated acids. Finally, the elegant relation one to another of the above features may be emphasized.

SUMMARY

1. Milk and depot fats derived from the Indian buffalo have been analyzed for component fatty acids and glycerides.

2. An inverse relationship between the lower acids and oleic acid has been shown to exist in the milk fats. Cotton-seed feeding results in a milk fat of high stearic and low palmitic acid content.

3. Two types of depot fat have been encountered —of high and low palmitic content respectively. An inverse stearic-palmitic balance is postulated for these fats in contrast to the inverse stearic-oleic relationship in western tallows.

4. Indian animal fats are shown to be characterized by the following features: high saturation, a species difference of 3 units between the iodine values for both the milk and depot fats of cows and buffaloes, a lower content of fully saturated glycerides corresponding to any fixed percentage of total saturated acids than in western fats, higher

Table 4. *Total saturated acids, fully-saturated glycerides and palmitic acid contents of Indian animal fats.*

Milk fats	Notation in Fig. 1	(As % by mol.) Observer	Total saturated acids	Fully-saturated glycerides	Palmitic acid content
Buffalo	1	Present sample 1	74·9	41·7	31·9
,,	2	,, ,, 3	62·9	24·3	25·1
,,	3	Bhattacharya & Hilditch (1931)	70·1	34·3	28·7
Cow	4	,, ,, ,,	67·9	33·7	26·8
Sheep	5	Dhingra (1933)	74·6	36·8	20·4
Goat	6	,, ,,	74·6	39·3	21·5
Camel	7	Dhingra (1934)	62·6	25·6	28·3
Depot fats					
Cow	*a*	Hilditch & Murti (1940)	72·9	35·9	40·8
,,	*b*	,, ,, ,,	67·5	28·3	33·4
Goat	*c*	Dhingra & Sharma (1938)	63·1	29·2	27·0
Buffalo	*d*	Present sample A	69·5	32·5	33·4
,,	*e*	,, ,, B	73·9	37·2	45·6

association ratios, and a higher, though still normally constant, proportion of mono-'oleo' disaturated glycerides.

We wish to express our grateful thanks to Prof. V. Subrahmanyan for his encouragement during the progress of these investigations.

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Indigoid Pigments Derived from a Pathological Urine

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WITH AN ADDENDUM ON

The Spectral Absorption of the Pigments

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(*Received* 23 *May* 1946)

This communication describes the isolation and identification of the indigoid pigments indirubin and indigotin from the acid-treated urine of a case of sprue. A relation between pigment production, severity of symptoms and the level of protein intake in the diet was observed.

MATERIAL

In the course of a study supervised by Wing-Commander T. F. Macrae of the urinary riboflavin excretion of cases of sprue among R.A.F. personnel, it was noticed that after autoclaving the urine for 15 min. at 120 lb. pressure in the presence of dilute hydrochloric acid (3·25 ml. of 2N-HCl to 25 ml. urine), adjusting to pH 4·0 and then shaking with chloroform, this solvent was occasionally coloured deep purplish red. A patient was given an experimental diet, rich in protein, for a preliminary period of 3 days and then for an experimental period of 5 days. The urine was collected during the experimental period, that is from the 4th to the 8th day after the commencement of the protein-rich diet. During the period of collection of urine the quantity of urinary pigment extractable by chloroform after autoclaving increased markedly and at the same time the patient's condition deteriorated. Specimens of the pigmented chloroform extracts were submitted to me for an opinion concerning the nature of the colouring matter, and this formed the starting point of the present investigation.

Out of ten patients given the experimental diet, the urines of at least five yielded the red chloroform-soluble pigment. It cannot be stated with certainty that any member of the group was negative in this respect, since the phenomenon in question was only observed incidentally and was not a primary object of the investigation.

METHODS AND RESULTS

Spectral characteristics and quantitative determination of the crude pigment. The colour of the washed and filtered crude chloroform extracts was a reddish purple. The spectral absorption in the visible region was determined by means of the Pulfrich step-photometer, with the set of Zeiss filters supplied with the instrument. A marked absorption band